Effect of Adsorption of Succinic Acid on the Formation of Amorphous Calcium Phosphate

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Adsorption of dissolved succinic acid on the formation of ACP and its stability were investigated in the synthetic process of ACP by the reaction of $CaCl_2$-$(NH_4)_2$HPO$_4$-$C_4H_6O_4$-NH$_4$OH. The crystallization of ACP to HAp and amount of succinic acid adsorbed were affected by some conditions such as initial pH, temperature, aging time and concentration of succinic acid in the mother liquor. The largest amount of succinic acid was adsorbed to ACP when the ACP was produced at 0°C, initial pH 10 and concentration of succinic acid 6.0mass% and aged at 25°C for 3h. From the result of infrared spectra of the ACP which adsorbed succinic acid, two peaks due to carboxylic acid were newly found at 1400cm^{-1} and 1550cm^{-1}. These peaks were not found from the result for a mixture of ACP and succinic acid. The adsorbed succinic acid was decomposed at about 480°C and ACP was crystallized to Ca$_3$(PO$_4$)$_2$ at above 600°C by heating.

1. Introduction

The minerals that constitute hard tissues of animals fall into two general categories, phosphates and carbonates, most of which are calcium compounds.[1] In particular, calcium phosphate represents the major portion of the mineral compounds that constitute hard tissues in a living body, such as those for bones and teeth. It has the form of a complex with collagen, a hard protein, and is known to have hydroxyapatite (Ca$_{10}$(PO$_4$)$_6$(OH)$_2$, HAp) as its major component.

HAp has recently been attracting attention as a material compatible with a living body. However, artificial HAp cannot be as crystalline as is natural counterpart found in a living body, although synthesized under living conditions.[2] It is accepted that amorphous calcium phosphate (ACP) is generally formed during the initial stage of HAp synthesis and is eventually transformed into HAp of low crystallinity.[3]

The authors have been conducting a series of investigations, expecting that these amorphous calcium compounds may express new functions.[4-12] They are particularly interested in basic aspects of ACP, such as its compositions, structures, properties, stability, and crystallization for morphological control to transform it into various calcium phosphates.[6-8] Having a large surface area, ACP may find wide applications as a material compatible with a living body through adsorption of organic and amino acids and through formation of composites with them. Many researchers have been discussing crystalline calcium phosphates, particularly their interactions with organic compounds.[13-18] The effects of organic compounds on the formation rate of ACP have been discussed,[3] but few

researchers have addressed the effects of their adsorption.

Glycin as one of the amino acids that constitute collagen, a fibrous protein, is formed from amino acid taken into a living body from the outside. It is then synthesized by the glycolysis process after undergoing the tricarboxylic acid (TCA) cycle to be transformed into various organic acids, such as malic, citric, and succinic acids, as the intermediates. In connection with the intermediates of these cycles, some researchers prepare hardened bodies by the reactions of calcium phosphate, such as tricalcium phosphate (Ca$_3$(PO$_4$)$_2$, TCP) and tetracalcium phosphate (Ca$_4$(PO$_4$)$_2$O), with organic acid, such as citric and succinic acids.[20-21] In particular, there has been no detailed discussion of the composites of succinic acid as a dicarboxylic acid with octacalcium phosphate (Ca$_8$H$_2$(PO$_4$)$_6$·5H$_2$O, OCP).[22-23]

In this study, the authors have prepared ACP in the presence of dissolved succinic acid, as one of the intermediates for glycin, to investigate adsorption of succinic acid on ACP, stability of ACP in the solution when it adsorbs succinic acid, and its stability under heat, noting ACP formed as the precursor for minerals compatible with a living body, in order to understand the basic aspects of the ACP-protein composites.

2. Starting Materials and Experimental Procedure

2.1. Starting Materials

The reagents used for the synthesis of ACP adsorbing succinic acid were special reagent grade, unhydrous calcium chloride (CaCl$_2$), diammonium hydrogen phosphate ((NH$_4$)$_2$HPO$_4$), and first-class reagent grade ammonia water (NH$_4$OH), all supplied by Kanto Kagaku Ltd.

In addition, reagent grade HAp (supplied by Nakarai Tesuku Ltd., 100 to 200meshes) for column chromatography was also used for comparison.

2.2. Experimental Procedure

ACP which adsorbed succinic acid was synthesized by the reactions in an aqueous solution of CaCl$_2$, (NH$_4$)$_2$HPO$_4$, C$_4$H$_6$O$_4$, and NH$_4$OH. First, a (NH$_4$)$_2$HPO$_4$ solution (1.0 × 10^{-1}mol·dm^{-3}) was added with powdered succinic acid to obtain a given concentration (0 to 6.0mass%) and with a 15N NH$_4$OH solution to control pH level during the synthesis process. When added rapidly with a CaCl$_2$ solution (1.67 × 10^{-1}mol·dm^{-3}), this solution turned into a gel-like substance to have a Ca/P atomic ratio of 1.67, where the volume of the CaCl$_2$ solution was set to the same level as that of the NH$_4$OH solution, and reaction temperature was controlled at 0°C. The gel-like substance thus formed was aged by allowing it to stand in the mother liquor at 0° to 40°C for 0 to 24h, and was then filtered by a glass filter (G5). It was washed with ammonia

water (kept at the same pH level as that used for the synthesis) and acetone, treated with silica gel to remove moisture, and finally dried in air to prepare ACP which adsorbed succinic acid.

The methods used for characterization of the products were powder X-ray diffractometry (Cu Kα, 30kV, 15mA), thermal analyses (differential thermal analysis, thermobalance, TG-DTA), infrared absorption spectrometry (KBr method), ion chromatography, and measurement of specific surface area. The product compositions were determined by chemical analysis, and quantities of phosphorus and succinic acid adsorbed on the products by ion chromatography (column: Toa Denpa Kogyo Ltd.'s PCI-305S).

The green compact of ACP carrying succinic acid was prepared by the following procedure: accurately measured 0.3g quantities of ACP, HAp and ACP carrying succinic acid were each maintained at 125kgf·cm⁻² for a given time (2 to 60min) in a mold, used to prepare the tablet for infrared spectrometry, by a hydraulic pump, in order to prepare a compact disc, 1.0cm in diameter and 0.3cm thick. The strength of this disc in the thickness direction (hereinafter referred to as compressive strength) was determined, as a measure for compressive strength, by a Kiya type hardness meter (Fujiwara Seisakusho Ltd., No.1748886), which is used to measure strength of medicine tablets.

3. Results and Discussion

3.1. Effects of Dissolved Succinic Acid on Formation of ACP

The authors have discussed that ACP having a Ca/P atomic ratio in a wide range from 1.25 to 1.55 can be synthesized by the hydrolysis in which ammonia water is rapidly added drop by drop to a monohydrogen calcium phosphate (Ca(H$_2$PO$_4$)$_2$ · H$_2$O, MCP) solution.[6-8,24] On the other hand, the reaction of CaCl$_2$ with (NH$_4$)$_2$HPO$_4$ in a solution by rapid mixing is discussed as the method to synthesize ACP having a high Ca/P ratio.[25-28] In this study, the latter method was adopted to synthesize ACP.

As a result, the ACP product had an atomic Ca/P ratio of 1.65 at an initial pH level of 10, demonstrating that ACP having a composition close to the theoretical HAp composition (atomic Ca/P ratio: 1.67) can be synthesized. It is confirmed that ACP is generally transformed into various calcium phosphate compounds as intermediates, depending on the type of aqueous solution in which it is aged, its pH and temperature levels, and type of organic additive used, and eventually crystallized into more stable HAp.[3,29] In this study, ACP formed was kept as it was in the mother liquor in order to investigate the effects of succinic acid on crystallization of ACP. Figure 1 illustrates the effects of aging time on crystallization of ACP formed in the presence of dissolved succinic acid as the results of X-ray diffraction analysis. ACP remained amorphous during the initial aging stage at up to 3h under the conditions of succinic acid concentration: 6.0mass%, initial pH level: 10, and aging temperature: 25°C, because the related samples exhibit no diffraction peaks but the broad patterns characteristic of amorphous state, as shown in Figs.1(a) and (b). On the other hand, when aging time increased to 6h or more, the diffraction peaks emerge at around 2θ = 25.8° and 32.0°C, as shown in Figs.1(c) and (d), indicating crystallization of ACP into HAp of low crystallinity. ACP was crystallized faster in the absence of succinic acid because of

the formation of HAp of relatively high crystallinity in 24h (Fig.1(e)). It is thus confirmed that succinic acid works to retard the crystallization, keeping ACP amorphous for 3h during the aging process, which is then crystallized into HAp of low crystallinity.

Next, the effects of succinic acid concentration on crystallization of ACP were investigated, noting the X-ray diffraction pattern at 2θ = 32.0°C, relevant to the (112) plane. The results are provided in Fig.2. In the absence of succinic acid, ACP remains amorphous for the first 2h during the aging process.
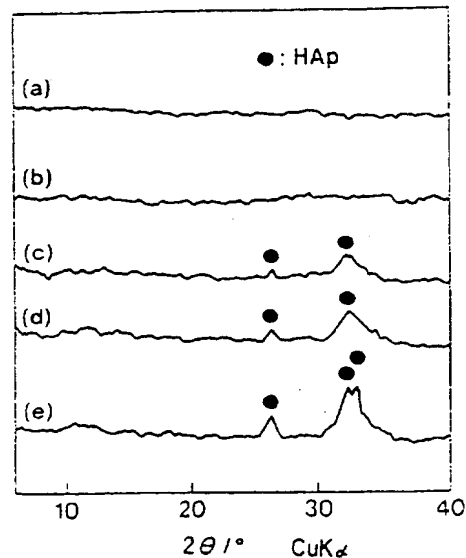

Fig. 1.   X-ray diffraction patterns for crystallization process of amorphous calcium phosphate in succinic acid solution.
Temperature (°C): 25, initial pH: 10, concentration of succinic acid (mass%): 6.0, time (h): (a) 1, (b) 3, (c) 6, (d) 24, (e) 24 (non-succinic acid).
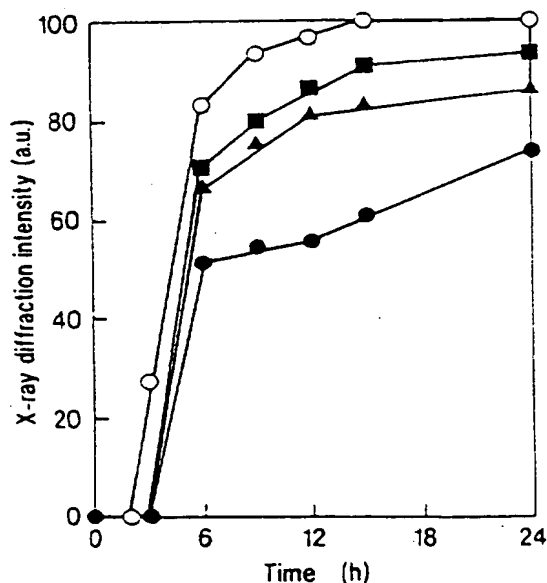

Fig. 2.   Effect of aging time on crystallization of amorphous calcium phosphate in succinic acid solution.
Temperature (°C): 25, initial pH: 10, concentration of succinic acid (mass%): ■ 0.4, ▲ 2.0, ● 6.0, ○ non-succinic acid.
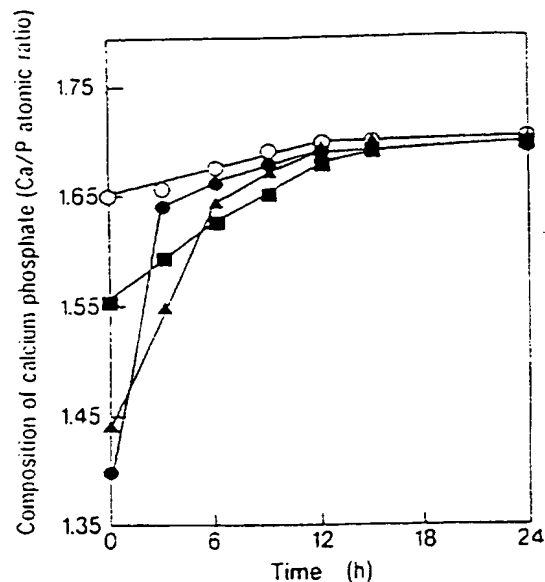
Fig. 3. Effect of aging time on composition of amorphous calcium phosphate in succinic acid solution.
Temperature (°C): 25. initial pH: 10. concentration of succinic acid (mass%): ■ 0.4, ▲ 2.0, ● 6.0, ○ non-succinic acid.
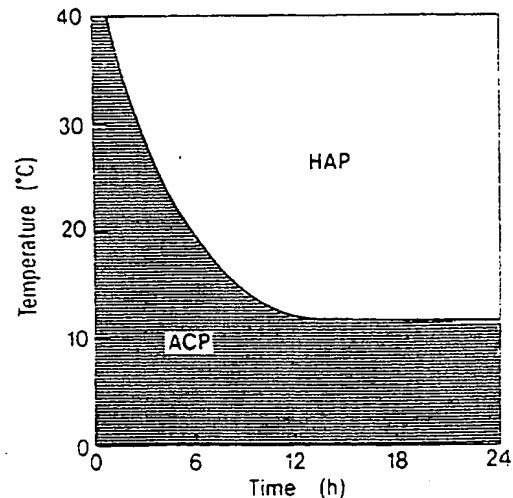

Fig. 4. Effect of aging time on stable region of amorphous calcium phosphate in succinic acid solution.
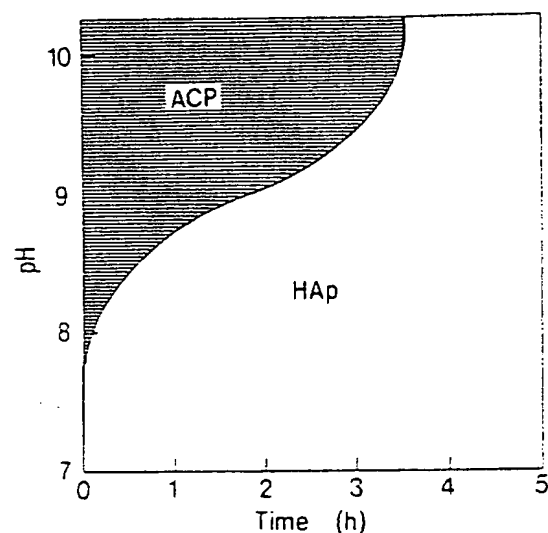Initial pH: 10. concentration of succinic acid (mass%): 6.0.

Fig. 5. Effect of initial pH on stable region of amorphous calcium phosphate in succinic acid solution.
Temperature (°C): 25. concentration of succinic acid (mass%): 6.0.

but it remains amorphous for 3h in the presence of succinic acid. It is also noted that HAp's X-ray diffraction intensity decreases as succinic acid concentration increases.

Figure 3 shows the atomic Ca/P ratio of ACP changing with aging time. The ratio is 1.65 in the absence of dissolved succinic acid immediately after the reaction process starts. whereas it successively decreases from 1.55 to 1.44 and 1.37 in the presence of the acid as its concentration increases. This conceivably results from the reactions of succinic acid during the initial stage with Ca^{2+} to form a soluble complex. However. atomic Ca/P moves towards the level of 1.67 that represents the theoretical HAp's composition. as aging time increases. irrespective of succinic acid concentration.

Figure 4 presents the X-ray diffraction analysis results for the effects of aging temperature on crystallization of ACP formed under the conditions of succinic acid concentration: 6.0mass% and initial pH level: 10. Increasing aging temperature tends to narrow the region in which ACP exists stably. indicating that ACP is more stable at a lower aging temperature.

Figure 5 illustrates the effects of initial pH level on crystallization of ACP formed in the presence of dissolved succinic acid. It is confirmed that HAp is present in the system immediately after the reaction process starts at a low initial pH level of 7. under the conditions of succinic acid concentration: 6.0mass% and aging temperature: 25°C. Increasing initial pH level broadens the region in which ACP stably exists; it remains amorphous for about 3h when initial pH level increases to around 10. It is discussed that crystallization of ACP is effectively controlled when a solution of high pH level is used for its synthesis.[3] and similar trends are observed in the presence of dissolved succinic acid.

3.2. Adsorption of Dissolved Succinic Acid on ACP

Figure 6 shows the infrared spectral patterns of ACP on which succinic acid is adsorbed. Comparing patterns (c) for

ACP on which succinic acid is adsorbed with those of (b) for the mixture of succinic acid and ACP. the former has the absorption spectra at 1550 and 1400cm⁻¹, relevant to carboxylates. which the latter does not have.[22,23] These spectra were also observed with the sample thoroughly washed with the NH_4OH solution with the adjusted pH for the synthesis. indicating that succinic acid is fast adsorbed on ACP.

Figure 7 illustrates the effects of succinic acid concentration and aging time on the quantity of succinic acid adsorbed on ACP under the conditions of aging temperature: 25°C and initial pH level: 10. Also provided for comparison are the results in which HAp for column chromatography is placed in a succinic acid solution of a given concentration. The quantity of succinic acid adsorbed on ACP tends to increase as succinic acid concentration increases. The quantity attains a maximum at an aging time of 3h. irrespective of succinic acid concentra-
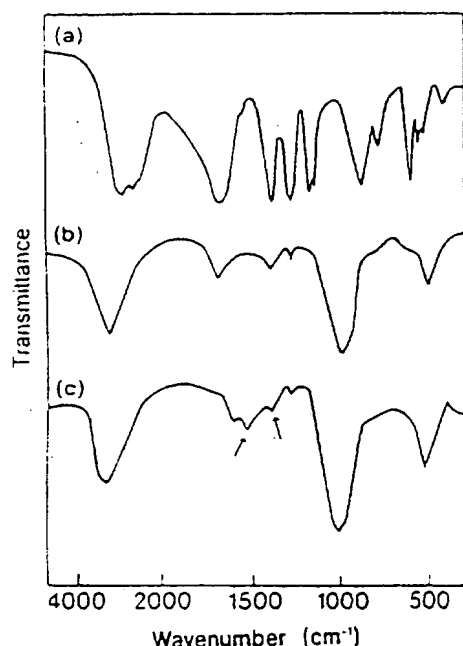
Fig. 6. Infrared spectra of amorphous calcium phosphate with adsorbed succinic acid and raw sample.

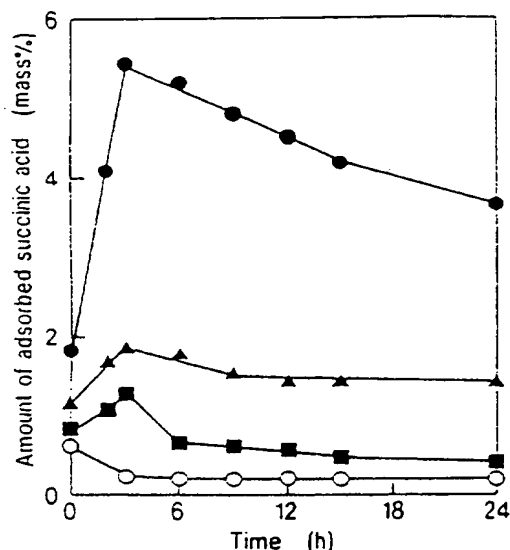(a) succinic acid, (b) ACP + succinic acid, (c) succinic acid adsorbed on ACP (5.5mass%).

Fig. 7. Effect of concentration of succinic acid on amount of succinic acid adsorbed on amorphous calcium phosphate.

Temperature (°C): 25, initial pH: 10, concentration of succinic acid (mass%): ■ 0.4, ▲ 2.0, ● 6.0 (ACP), ○ 6.0 (HAp).

tion, exhibiting the highest level of 5.5% at an acid concentration of 6.0mass%. It decreases with time after an elapse of 3h, conceivably resulting from progress of the crystallization of ACP to HAp. The largest quantity of succinic acid adsorbed on HAp is approximately 0.5% when it is placed in a succinic acid solution (liquid-phase adsorption).

Figure 8 illustrates the effects of aging temperature on adsorption of succinic acid on ACP. Adsorption of the acid is
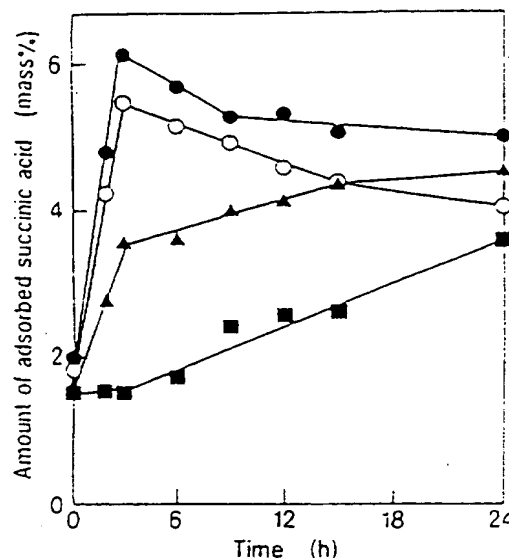

Fig. 8. Effect of aging temperature on amount of succinic acid adsorbed on amorphous calcium phosphate.

Initial pH: 10, concentration of succinic acid (mass%): 6.0, temperature (°C): ■ 0, ▲ 10, ○ 25, ● 30.

retarded at low aging temperature levels of 0° and 10°C at a synthesis temperature of 0°C; quantities of the acid adsorbed on ACP are around 3.5 and 4.0% at respective aging temperature levels, even when aging time is increased to 24h. However, adsorption of succinic acid is rapidly accelerated as aging temperature is increased to 25° and 30°C; e.g., the adsorbed quantity increases to 5.5% under the conditions of 25°C and 3h, and to the highest observed level of 6.0% under the conditions of 30°C and 2h. It is also noted, as shown in Fig.4, that the stability of ACP is very sensitive to temperature. ACP remains amorphous at a low aging temperature for 24h, from which it is judged that quantity of succinic acid adsorbed will further increase with time.

It is thus essential, in order to increase quantity of succinic acid adsorbed on ACP, to increase succinic acid concentration as much as possible and to keep aging temperature in a range from 25° to 30°C. It is also observed, when ACP adsorbing succinic acid is further aged in the mother liquor, that HAp adsorbing succinic acid through the liquid-phase adsorption can be prepared.

3.3. Thermal Stability of ACP Adsorbing Succinic Acid

Thermal analysis was performed for ACP which adsorbed succinic acid. Figure 9(A) is the TG-DTA curve of ACP synthesized under the conditions of aging temperature: 25°C and initial pH level: 10, where ACP adsorbs 5.5% of succinic acid. Figure 9(B) and (C) present the infrared absorption and X-ray diffraction patterns at temperature levels of (a) through (d), shown in (A). Comparing the sample heated to 400°C (b) with the unheated one (a), the former has the diminished absorption spectra at around 3600 and $1640cm^{-1}$, which are relevant to the Lk and vibration of OH^-.[10] There are two types of water molecules present in ACP, adsorbed water and water of hydration.[24,30] Therefore, the endothermic peak found in the DTA curve at around 100°C results from elimination of water of hydration. The sample heated up to 400°C (b) has the spectra at around 1550 and $1400cm^{-1}$, which are relevant to carboxylates. They are no longer found with the sample
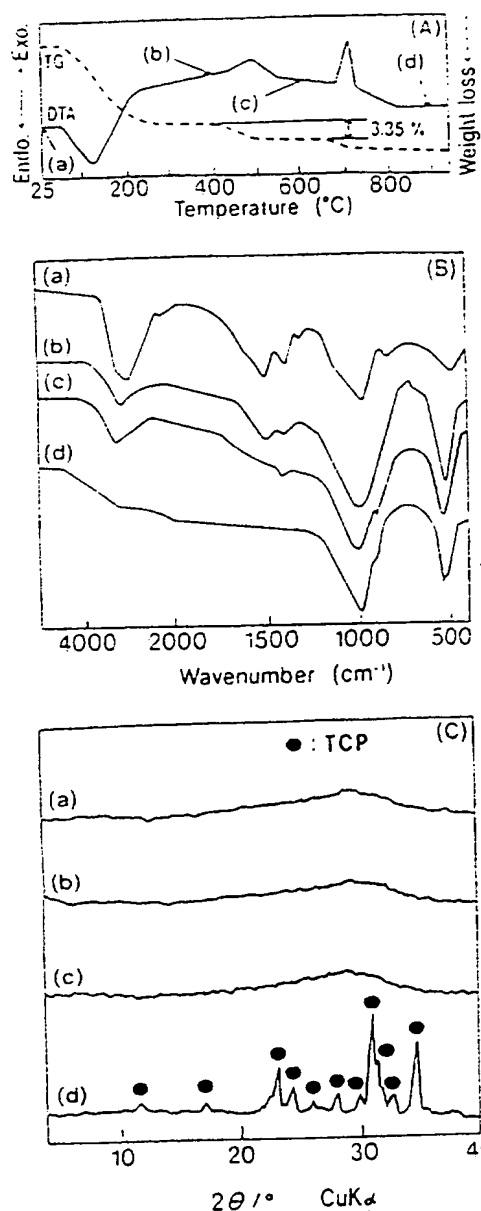
Fig. 9. Thermal decomposition process of amorphous calcium phosphate with adsorbed succinic acid (5.5 mass%).

(A) TG-DTA curve. (B) infrared spectra. (C) X-ray diffraction patterns.

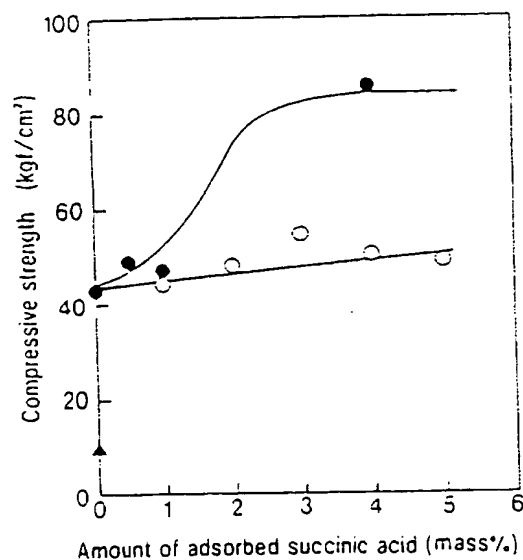Temperature (°C): (a) 25. (b) 400. (c) 600. (d) 900.

Fig. 10. Compressive strength for powder-compacts of amorphous calcium phosphate with adsorbed succinic acid.

● succinic acid adsorbed on ACP. ○ ACP – succinic acid. ▲ HAp.

3.4. Strength of ACP Compact Adsorbing Succinic Acid

HAp and TCP. now used as the materials compatible with a living body. have been molded into desired shapes under elevated temperature and pressure, except when they are used in the form of particles or granules because of their low chemical reactivity.[31] When ACP that adsorbs an organic acid is molded into a compact. that the organic acid works as a binder in a living body. it is expected to make ACP stronger and highly compatible with a living body.

Compressive strength was measured for a compact of ACP that adsorbs succinic acid. The results are provided in Fig.10. which also shows the strength of the compacts of HAp and ACP itself. for comparison. Compressive strength of the ACP compact is 43kgf/cm^2, whereas that of the HAp is lower at 10kgf/cm^2. One of the reasons for the higher strength of the former compact is the finer particles agglomerating into the compact. knowing that the compacts of ACP. HAp, and ACP adsorbing 5.5% of succinic acid have specific surface areas of 60. 38, and 75m^2/g, respectively. On the other hand. compressive strength of the compact of ACP that adsorbs succinic acid increases as the quantity of the acid adsorbed increases. to 80kgf/cm^2 at the highest.

These results indicate that succinic acid adsorbed on ACP works as a binder. making it possible to realize the compact highly compatible with a living body.

4. Conclusion

Amorphous calcium phosphate was prepared by the reactions in an aqueous solution of $CaCl_2$, $(NH_4)_2HPO_4$. $C_4H_6O_4$, and NH_4OH in order to investigate the effects of dissolved succinic acid adsorbed on phosphate and its stability on formation of phosphate. The largest quantity of succinic acid can be most effectively adsorbed on ACP when it is synthesized under the conditions of temperature: 0°C and initial pH level: 10 to have 6.0mass% of the acid, and then aged at room temperature (25°C).

heated up to 600°C (c). which suggests that the exothermic peak found at around 480°C results from combustion of the decomposed carboxylates.

There is a sharp exothermic peak at around 700°C. The X-ray diffraction patterns (B) indicate that the sample heated up to 600°C (c) has the broad patterns characteristic of amorphous substance: however. the one heated to 900°C (d) has the peaks relevant to tricalcium phosphate ($Ca_3(PO_4)_2$, TCP). from which it is considered that the sharp peak results from the crystallization of ACP to TCP.

It is also confirmed that the mixture of ACP and succinic acid has the exothermic peak resulting from decomposition of succinic acid at around 350°C. indicating that ACP which adsorbs succinic acid is stable at up to around 480°C.

It is confirmed that succinic acid adsorbed on ACP is decomposed and combusted at 480°C or higher and that ACP is transformed into $Ca_3(PO_4)_2$ at 600°C or higher. These results indicate that succinic acid adsorbed on ACP is stable at up to around 480°C, and that the acid is fast adsorbed on the ACP surfaces. The compact of ACP that adsorbs succinic acid exhibits a compressive strength of 80kgf/cm², approximately twice that of the compacts of ACP or HAp by itself, thanks to adsorbed succinic acid that serves as a binder.

At present, the studies on the materials compatible with a living body are centered around those for HAp. However, ACP which is more chemically active than HAp may be applicable to the materials having affinity for a living body by treating it to be adsorbed with an organic or amino acid before it is molded into a compact.

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